



Phylogenetic Analysis of Vertebrate Lysozyme

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ABSTRACT

Lysozyme is a naturally occurring enzyme found in bodily secretions such as tears, saliva, and milk. It functions as an antimicrobial agent by cleaving the peptidoglycan component of bacterial cell walls, which leads to cell death. In present study we chose 20 Vertebrate Species including 1 amphibian, 3 actinopterygii, 7 aves and 9 mammals. Amino acid sequences of Lysozyme from 20 vertebrates were derived from National Center for Biotechnology Information database. Phylogenetic analysis and estimation were performed using MEGA program, and neighbor-joining tree was constructed by this software. In this computational study, the phylogenetic tree of Lysozyme has been investigated. Therefore, dendrograms of lysozyme were depicted. From the result of present phylogenetic study of vertebrate Lysozyme, it concluded that the amino acid sequences among different vertebrate species shows slight to moderate differences without affecting its functions.

Key words: Lysozyme, Antimicrobial activity, MEGA, Phylogeny, Vertebrate

INTRODUCTION

Lysozyme is an antimicrobial enzyme which has been widely found across the animal kingdom as a natural bactericide. Lysozyme was first discovered by Alexander Fleming (1922) in the nasal mucus and subsequently purified from various plant, animal, microbial (bacteria, virus and fungi) materials (Masschalck and Michiels, 2003; Xue *et al.*, 2004; Parisien *et al.*, 2007; Fleming, 1922). Lysozyme (enzyme commission number 3.2.1.17) is also called as N-acetylmuramidase or muramidase, lysozyme is a hydrolyse-type enzyme that catalyses the breakdown of peptidoglycan polymers of bacterial cell wall at the 1-4 bond between N-acetylmuramic (NAM) acid and N-acetylglucosamine (NAG) residues, thereby lysing sensitive bacteria. *In vitro*, lysozyme is generally considered effective against some Gram-positive bacteria, but ineffective against Gram-negative bacteria (Cunningham *et al.*, 1991)

Although lysozyme is important for driving a pro-inflammatory response, lysozyme also plays a role in limiting inflammation systemically, resulting in decreased inflammatory driven pathology (Nash *et al.*, 2006; Ganz *et al.*, 2003) Due to its bacteriostatic, bactericidal, and antiviral properties, lysozyme is currently used as an active pharmaceutical ingredient in medicine and veterinary medicine as well as in the food industry (Leśnierowski and Kijowski, 2007; Aminlari *et al.*, 2014). Lysozyme represents one of the most-studied biopreservatives for antimicrobial packaging application. (Silveti *et al.*, 2017)

By applying a specific evolutionary model to explain the amino acid substitutions observed in the multiple sequence alignment, the evolutionary distances between all pairs of proteins can be computed. This evolutionary distance, which reflects the expected mean number of changes per site that have occurred since two sequences diverged from their common ancestor, is used by the so-called distance-methods for phylogenetic inference. One such method is Neighbor Joining (NJ) (Gabaldón, 2007). NJ constitutes a good and fast heuristic algorithm that estimates the “minimal evolution” tree, a phylogenetic tree which minimizes the sum of the lengths (evolutionary distances) of all its branches (Saitou, 1987).

In present study we chose 20 Vertebrate Species including 1 amphibian, 3 actinopterygii, 7 aves and 9 mammals and performed phylogenetic analysis on lysozyme to explore the study of evolutionary relations among groups of organisms. Establishing a relation between structure, function and evolution of this protein is of paramount

importance because it would provide better understanding of further possible mechanisms that the protein involves in.

MATERIAL AND METHOD

Amino acids sequences for the lysozyme protein of 20 vertebrate species were taken from National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>). Alignments were applied in order to build a neighbor-joining phylogenetic tree using the Mega program. A phylogenetic tree was saved and analyzed. Constructed phylogenetic tree is depicted in figure 2

table 1: accession numbers of ncbi entries for lysozyme in different vertebrate species

Accession numbers	Name of vertebrate species
CAA32175.1	<i>Homosepian</i>
AAA41551.1	<i>Rattus norvegicus</i>
AIE13825.1	<i>Canis lupus</i>
ACL81750.1	<i>Gallus gallus</i>
AIA09919.1	<i>Xenopus laevis</i>
CAA42084.1	<i>Oncorhynchus mykiss</i>
AAC37312.1	<i>Bos taurus</i>
AAB16862.1	<i>Sus scrofa</i>
AAA73935.1	<i>Opisthocomus hoazin</i>
AHA61788.1	<i>Capra aegagrus hircus</i>
ACE80211.1	<i>Scophthalmus maximus</i>
AIZ00421.1	<i>Anguilla japonica</i>
AIN76235.1	<i>Oryctolagus cuniculus</i>
AHI42590.1	<i>Ovis ammon</i>
ACL81752.1	<i>Bambusicola thoracicus</i>
AAB97109.1	<i>Trichosurus vulpecula</i>
AAB31830.1	<i>Syrmaticus soemmerringii</i>
PKK17361.1	<i>Columba livia</i>
ACL81754.1	<i>Francolinus pondicerianus interpositus</i>
AAB20837.1	<i>Lophura leucomelanos</i>

RESULTS AND DISCUSSION

The lysozyme protein sequences of 20 vertebrate species were aligned and analyzed. The phylogenetic analysis was carried out by using distance method and clustering by UPGMA (Unweighted Pair Group Method with Arithmetic Mean). A dendrogram of Lysozyme drawn by using MEGA7 multiple sequence alignment of the Lysozyme proteins is depicted in Figure1 and constructed phylogenetic tree is depicted in Figure 2.

Species/Abbrv	
1. Bos taurus	MKALIIILGFLFLSVAVDQKVFERCEELARTLKKLGLDGYKGVSLA
2. Oncorhynchus mykiss	MRAVVVLLLVAVASAKVYDRCEELARALKASGMDGYAGNSLPNWV
3. Gallus gallus	MRSLLILVLCFLPLAALGKVFGRCELAAMKRHGLDNYRGYSLG
4. Sus scrofa	MKTLLVLAALLLSVSVDAKVYDRCEEFARILKKSMDGYRQVSLA
5. Homo sapiens	KVFERCEELARTLKRLLGMDGYRGIISLANWMCCLAKWESGYNTRATN
6. Rattus norvegicus	MKALLVLGFLLLSASVDAKIYERCFARTLKRNGMSGYVGVSLA
7. Canis lupus familiaris	MKTLLFLGLLLSITVQKIFERCEELARTLKNLGLAGYKGVSLA
8. Xenopus laevis	MKLFERCELAGTMMKKMGLDGYRQYSLPNWVCTAFFESSFYTDRT
9. Opisthocomus hoazin	MLFFGFLLAFLSAVPGTEGEIIPRCELVKILREHGFEGFECTTI
10. Capra hircus	MKALIIILGFLFLSVAVDQKVFERCEELARTLKKLGLDGYKGVSLA
11. Anguilla japonica	MRALVFLLVAVASAKVYERCEELARTLKAAGMDGYRQVSLGQWV
12. Ovis ammon	MKALIIILGFLLLSASVDAKVYERCEELARTLKRFGMDGFRGISLA
13. Bambusicola thoracicus	MRSLLILVLCFLPLAALGKVFGRCELAAMKRHGLDNYRGYSLG
14. Trichosurus vulpecula	MKVLVLLGFIICSMAAHGKRMERCEEFARRIKQLHLDGYHQLSLA
15. Oryctolagus cuniculus	MKALLILGLLLSITVQKIYERCEELARTLKKLGLDGYKGVSLA
16. Columba livia	MRTSMLFVGLLIFLGLALPQIAKDIIPRCELVKILRRHGFEGF
17. copper pheasants	KVYGRCELAAMKRLLGLDNYRQYSLGNWVCAAKFESNFNTHATN
18. Scophthalmus maximus	MRCLLLLLVAVAGAKVYERCEELARLLKRNGMSNYRQYSLADWV
19. Lophura leucomelana	KVYGRCELAAMKRLLGLDNYRQYSLGNWVCAAKFESNFNTHATN
20. Francolinus pondicerianus interpositu	MRSLLILVLCFLPLAALGKVFGRCELAAMKRHGLDNYRGYSLG

Continued from Above

Species/Abbrv	
1. Bos taurus	NWLCCLTKWESSYNTKATNYPSSSESTDYGIFQINSKWWCNDGK
2. Onchorhynchus mykiss	CLSKWESSYNTQATNRRNTDGSTDYGIFQINSRYWCDDGRTPGA
3. Gallus gallus	NWVCAAKEFSNFNTQATNRRNTDGSTDYGIFQINSRWWCNDGRT
4. Sus scrofa	NWVCLAQWESDFNTKAINHNVCSTOYGFIFQINSRYWCNDGKTP
5. Homo sapiens	YNAGDRSTDYGFIFQINSRYWCNDGKTPGAVNACHLSCSALLQD
6. Rattus norvegicus	DWVCLAQHESNYNTQARNYNPGDQSTDYGIFQINSRYWCNDGK
7. Canis lupus familiaris	NWVCLAQWESNYNTRATNYPGSKSTDYGIFQINSRYWCNDGK
8. Xenopus laevis	NFNRCQNSTDYGILQINSRWWCNDGKTPRSHNACNINCRQLLS
9. Opisthocomus hoazin	ADWICLVQHESDYNTAEAYNNNGPSRDYGFIFQINSKYWCNDGKT
10. Capra hircus	NWLCCLTKWESSYNTKATNYPSSSESTDYGIFQINSKWWCNDGK
11. Anguilla japonica	CLARWESSYNTAATNRRNTDGSTDYGIFQINSRWWCNNGVITPSK
12. Ovis ammon	NWMCLARWESSYNTQATNYSNGDRSTDYGFIFQINSHWWCNDGKT
13. Bambusicola thoracicus	NWVCAAKEFSNFKTHATIRNTDGSTHYGILQINSRWWCNDGKT
14. Trichosurus vulpecula	NWVCLAQWESGFDTKATNYPGDDSTDYGILQINSHYWCDDGK
15. Oryctolagus cuniculus	NWMCLAQWESSYNTATNYPGDKSTDYGFIFQINSRYWCNDGK
16. Columba livia	VGKTVAQWVCLVKHESGYRTTAFNNNGPSRDYGFIFQINSKYWC
17. copper pheasants	RNTDGSTDYGILQINSRWWCNDGKTPGSRNLCNIPCSALLSSD
18. Scopthalmus maximus	CLSQWESSYNTATNRRNTDGSTDYGIFQINSRWWCNDGQTPTS
19. Lophura leucomelana	RNTDGSTDYGILQINSRWWCNDGKTPGSRNLCNIPCSALLSSD
20. Francolinus pondicerianus interpositu	NWVCAAKEFSNFKTHATIRNTDGSTYYGIFQINSRWWCNDGRT

Continued from Above

Species/Abbrv	
1. Bos taurus	TPNAVDDGCHVSCSELMENDIAKAVACAKHIVSEGGITAWVAWKS
2. Onchorhynchus mykiss	KNVCCIRCSQLLTADLTVAIRCAKRVVLDPNIGICAWVAWRHLCD
3. Gallus gallus	PCSRNLCNIPCSALLSSDITASVNCACKIIVSDGNGMNAWVAWRN
4. Sus scrofa	KAVNACHISCKVLLDDBLSDIECAKRVVDRPLGVKAWVAWRAH
5. Rattus norvegicus	TPRAKNACGIPCSALLODDITQAIQCAKRVVDRPQGIRAWVAWQ
6. Canis lupus familiaris	TPRAVNACHISCSALLODDITQAVACAKRVVSDPFGIRAWVAWR
7. Homo sapiens	NIADAVACAKRVVDRPQGIRAWVAWRNRCQNRDVRQYVQGCYV
8. Xenopus laevis	DDITQSVICAKRVVDRPQGMCAWVGRNHCGRDLSQWIKDCKL
9. Opisthocomus hoazin	SCAVDDGCHISCSSELMENDLEDDIKCAKKIARDAGLTPWYQWKN
10. Capra hircus	TPNAVDDGCHVSCSELMENDIAKAVACAKHIVSEGGITAWVAWKS
11. Anguilla japonica	NGCNISCSLLSSDIDISTAITCAKRVVDRPFGIRAWVAWRAHCEG
12. Ovis ammon	TPGAVNACHIPCSALLODDITQAVACAKRVVSDPQGIRAWVAWR
13. Bambusicola thoracicus	PCBRNLCNIPCSALLSSDITASVNCACKIIVSDGNGMNAWVAWRN
14. Trichosurus vulpecula	TPHAANECKVRCSELEDDLVKAVNCAKKIIVDQGGIRAWVAWRN
15. Oryctolagus cuniculus	TPRAVNACHIPCSALLKDDITQAVACAKRVVSDPQGIRAWVAWR
16. Columba livia	NDCKTRGSKNACHINCSKLRDDNIVDDIQCAKKIAREARGLTPW
17. copper pheasants	TIASVNCACKIIVSDGNGMNAWVAWRKRCKCTDQVNAWTRGCRLL
18. Scopthalmus maximus	NACGISCSALLTDDVGAALICAKHVVDRPFGIGAWVAWKRRCQV
19. Lophura leucomelana	ITASVNCACKIIVSDGNGMNAWVAWRNRCCKCTDQVSVWTRGCRLL
20. Francolinus pondicerianus interpositus	PCBRNLCNIPCSALLSSDITASVNCACKIIVSDGNGMNAWVAWRN

Continued from Above

Species/Abbrv	
1. Bos taurus	HCRDHDVSSYVQGGCTL---
2. Onchorhynchus mykiss	NQDLRSYVAGCGV-----
3. Gallus gallus	RCKGTDVQAWIRGCRLL---
4. Sus scrofa	CQNKDVSSQYIRGCKL---
5. Rattus norvegicus	RHCKNRDLSSQYIRNCGV---
6. Canis lupus familiaris	AHCENRDVSSQYVRNCGV---
7. Homo sapiens	-----
8. Xenopus laevis	DILL-----
9. Opisthocomus hoazin	HCEGRDLSSYVVKGG-----
10. Capra hircus	HCRDHDVSSYVEGCTL---
11. Anguilla japonica	QDVSQYIAGCGV-----
12. Ovis ammon	GHCQNDQLTSYIQGGCV---
13. Bambusicola thoracicus	RCKGTDVQAWIRGCRLL---
14. Trichosurus vulpecula	KCEGKDLGKYLEGCHL---
15. Oryctolagus cuniculus	NHCQNDQLTPYIQGGCV---
16. Columba livia	VAWKKYCGCKNLSSYVRGC
17. copper pheasants	-----
18. Scopthalmus maximus	QDLSSYVAGCGV-----
19. Lophura leucomelana	-----
20. Francolinus pondicerianus interpositus	RCKGTDVQAWIRGCRLL---

figure 1: multiple sequence alignment of lysozyme.

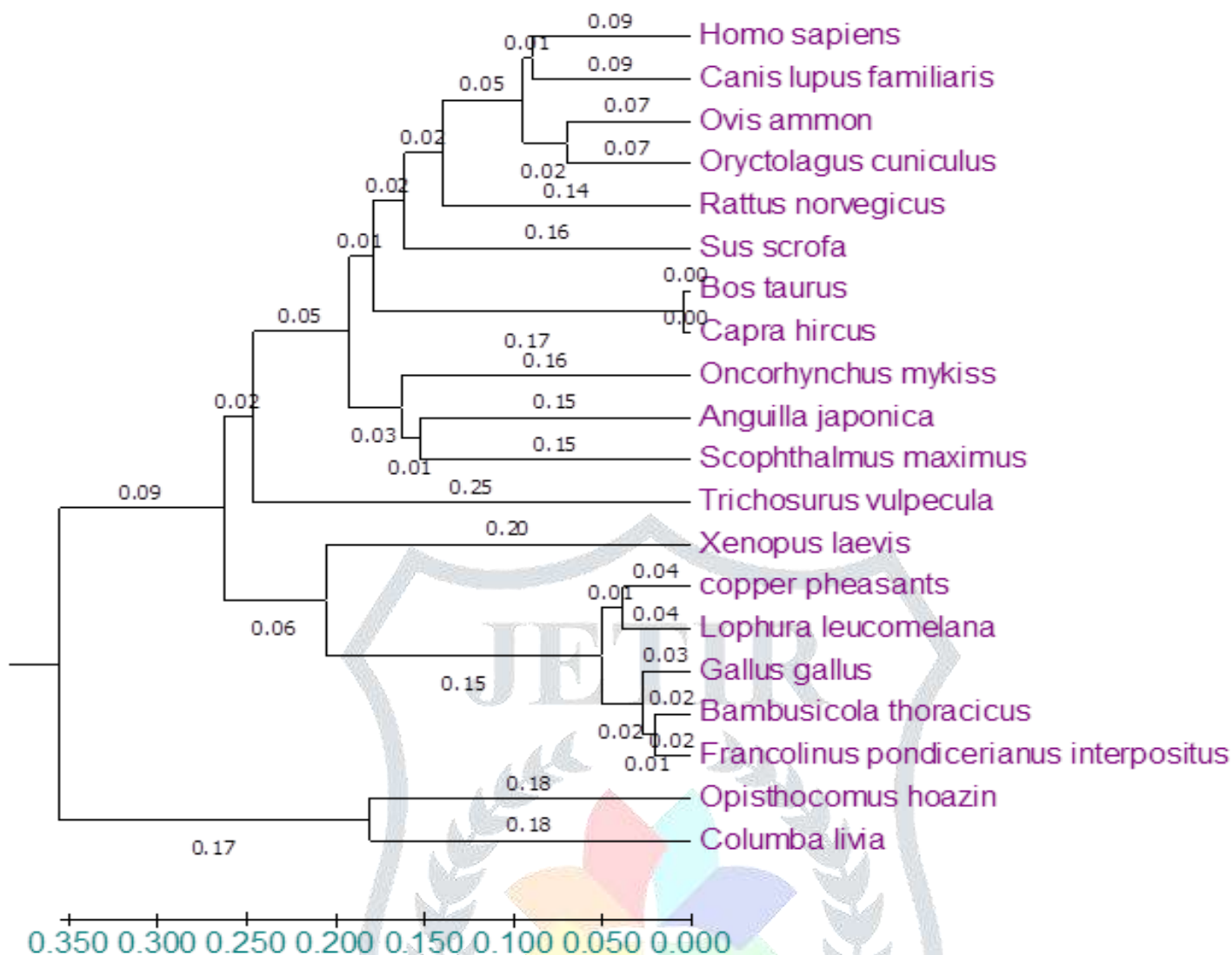


figure 2. phylogenetic tree of lysozyme in vertebrate species.

The phylogenetic tree reveals the formation of an outgroup comprising of *Xenopus laevis* (African clawed frog) and *Trichosurus vulpecula*. The sequences of *Homo sapiens* (Human), *Canis lupus familiaris* (gray wolf), *Ovis ammon* (mountain sheep), *Oryctolagus cuniculus* (European rabbit), *Rattus norvegicus* (brown rat) and *Sus scrofa* (wild boar) bear moderate similarities and also form a cluster.

The sequences of *Bos Taurus* (Cattle) and *Capra hircus* (domestic goat) are absolutely similar to each and form a cluster. The sequences of *Alligator* *Oncorhynchus mykiss* (rainbow trout), *Anguilla japonica* (Japanese eel), *Scophthalmus maximus* (turbot) show considerable similarity with each other and form a cluster. The sequences of *copper pheasants* (*Syrmaticus soemmerringii*) and *Lophura leucomelana* (kalij pheasant) are absolutely similar to each and form a cluster.

The sequences of *Gallus gallus* (red junglefowl), *Bambusicola thoracicus* (Chinese bamboo partridge), *Francolinus pondicerianus interpositus* shows Considerable similarity and also form a cluster. The sequences of *Opisthocomus hoazin* (hoatzin) and *Columba livia* (rock pigeon) are absolutely similar to each and form a cluster. The dendrogram based upon the protein sequences of Lysozyme Protein demonstrate the presence of 6 clusters among the 20 different animals. The similarity in the sequence among all the selected species are shown in the above dendrogram.

CONCLUSIONS

From the results and discussion of present phylogenetic analysis study of vertebrate Lysozyme, the amino acid sequences among different mammalian species show slight to moderate differences without affecting its of antimicrobial activity.

It also concluded from the phylogenetic tree analysis that, Lysozyme in different species also have strong to moderate sequence similarities among studied vertebrates.

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